TECHNICAL SUPPORT SECTION EFFICACY REVIEW - I

Disinfectants Branch

IN 10-22-85 OUT 12-20-85

Reviewed By Dennis G. Guse Date 12-20-85

EPA Reg. No. or File Symbol 1043-OR & 1043-OE

EPA Petition or EUP No. None

Date Division Received 09-12-85

Type Product Hospital (Veterinary) Disinfectant & FCS Sanitizer

Data Accession No(s). 259760 & 259758

Product Manager 32 (Castillo)

Product Name LpH-AG & LpH-SE

Company Name Vestal Laboratories

Submission Purpose New product applications: Same formulation

Type Formulation Liquid concentrate

Active Ingredient(s):	%
o-Phenylphenol	7.3
p-tert-Amylphenol	7.4

200.0 Introduction

200.1 Uses:

Both products are proposed as "one-step" cleaner-disinfectants, fungicides against pathogenic fungi, tuberculocides, and virucides on hard, non-food surfaces such as floors, walls, counters, tools, carts, and other equipment in food processing and service facilities, veterinary clinics, and animal research facilities. LpH-AG is additionally recommended for livestock and poultry premises.

Both products are also proposed as "one-step" cleaner-disinfectants and "two-step" cleaner-sanitizers for food-contact surfaces and equipment in food processing and service facilities, followed by a potable water rinse.

200.2 Background

Both products are identical in formulation.

201.0 Data Summary

The data submitted under Accession No. 259760 for LpH-AG (EPA File Symbol 1043-OR) and Accession No. 259758 for LpH-SE (EPA File Symbol 1043-OE) are the same.

201.1 Brief Description of Tests (Accession Nos. 259760 & 259758)

a. Basic Bacteriological Data (Disinfectant - Required Bacteria) and Microbiological Data (Disinfectant & Fungicide - Additional Bacterial and Fungal Pathogens). Tests conducted by Operators AD and JA, Vestal Laboratories, Inc., St. Louis, MO 63110, dated from 03-01-85 to 08-19-85. Report undated.

b. AOAC Methods of Analysis - Fungicidal Test, Tuberculocidal Test, and Germicidal & Detergent Sanitizer Test. Reports Terry Vigneault, Manager, Microbiological Laboratory Services, Northview Laboratories, Inc., Northbrook, IL 60062, dated 04-30-85 to 08-21-85.

c. Virucidal Tests. Reports by Dr. Herbert N. Prince, Director, Gibraltar Biological Laboratories, Inc., Fairfield, NJ 07006, dated 06-14-85 to 09-04-85.

201.2 Test Summaries

a. AOAC Use-Dilution Method

1. Modifications: Organic soil load (5% v/v blood serum added to microbial inoculum) and hard water (use-dilution made in 400 ppm $CaCO_3$ hard water). Control carrier viability determined after drying.

2. Samples: LpH-SE/LpH-AG, Batch Nos. 673-16C-I (02-11-85), 673-16C-II (02-26-85), and 673-16C-III (08-14-85).

3. Dilution: 1:256.

4. Exposure: 10 minutes at 20C.

5. Test Organisms: Staphylococcus aureus ATCC 6538 (PR 1:60), Salmonella choleraesuis ATCC 10708 (PR 1:80), Pseudomonas aeruginosa ATCC 15442 (PR 1:70 to 1:80), Acinetobacter calcoaceticus ATCC 19606 (PR 1:70), Citrobacter freundii ATCC 8090 (PR 1:90), Enterobacter aerogenes ATCC 13048 (PR 1:70), Enterobacter cloacae ATCC 23355 (PR 1:80), Escherichia coli ATCC 25922 (PR 1:80), Klebsiella pneumoniae ATCC 13883 (PR 1:80), Proteus mirabilis clinical isolate (PR 1:90), Proteus vulgaris ATCC 13315 (PR 1:90), Pseudomonas aeruginosa ATCC 27853 (PR 1:80), Salmonella typhi ATCC 6539 (PR 1:80), Salmonella typhimurium ATCC 14028 (PR 1:70), Serratia marcescens ATCC 8100 (PR 1:70), Shigella flexneri ATCC 12022 (PR 1:70), Shigella sonnei ATCC 25931 (PR 1:70), Staphylococcus aureus ATCC 25923 (PR 1:50), Staphylococcus epidermidis ATCC 12228 (PR 1:50), Streptococcus faecalis ATCC 19433 (PR 1:40), Streptococcus pyogenes ATCC 19615 (PR 1:70), Multiply (Methicillin)-Resistant Staphylococcus aureus (MRSA) clinical isolate(PR 1:60), Candida albicans clinical isolate (PR 1:70), and Candida parapsilosis clinical isolate (PR 1:60).

6. Subculture Medium/Neutralizer: Letheen broth or tryptic soy broth w/ letheen (bacteria) and Sabouraud dextrose broth w/letheen (fungi).

7. Incubation: 48 hours at 37C (per method).

8. Results:

Test Organism	Test Batch	Viable Count Per Carrier	Positive/Total Carriers
Staphylococcus aureus (6538)	I-(60-Day)	3.1×10^6	0/60
" " "	II	2.5×10^6	0/60
" " "	III-(60-Day)		0/60
Salmonella choleraesuis (10708)	I-(60-Day)	1.4×10^6	0/60
" " "	II-(60-Day)	1.1×10^6	0/60
" " "	III-(60-Day)		0/60
Pseudomonas aeruginosa (15442)	I-(60-Day)	3.6×10^6	0/60
" " "	II	6.5×10^6	0/60
" " "	III-(60-Day)		0/60
Acinetobacter calcoaceticus (19606)	II	4.7×10^6	0/10
" " "	III	9.2×10^5	0/10

Test Organism	Test Batch	Viable Count Per Carrier	Positive/Total Carriers
Citrobacter freundii (8090)	II	4.5×10^5	0/10
" " "	III	8.4×10^5	0/10
Enterobacter aerogenes (13048)	II	2.5×10^7	0/10
" " "	III	7.7×10^5	0/10
Enterobacter cloacae (23355)	II	2.5×10^7	0/10
" " "	III	1.1×10^7	0/10
Escherichia coli (25922)	II	8.2×10^6	0/10
" " "	III	1.6×10^7	0/10
Klebsiella pneumoniae (13883)	II	1.7×10^6	0/10
" " "	III	3.4×10^6	0/10
Proteus mirabilis (C.I.)	II	7.4×10^5	0/10
" "	III	4.9×10^5	0/10
Proteus vulgaris (13315)	II	3.4×10^6	0/10
" " "	III	2.1×10^6	0/10
Pseudomonas aeruginosa (27853)	II	5.2×10^6	0/10
" " "	III	4.5×10^6	0/10
Salmonella typhi (6539)	II	2.1×10^7	0/10
" " "	III	8.4×10^6	0/10
Salmonella typhimurium (14028)	II	1.2×10^7	0/10
" " "	III	1.3×10^6	0/10
Serratia marcescens (8100)	II	6.7×10^6	0/10
" " "	III	1.5×10^7	0/10
Shigella flexneri (12022)	II	1.1×10^6	0/10
" " "	III	6.7×10^5	0/10
Shigella sonnei (25931)	II	5.7×10^6	0/10
" " "	III	3.1×10^6	0/10
Staphylococcus aureus (25923)	II	6.0×10^6	0/10
" " "	III	1.5×10^6	0/10
Staphylococcus epidermidis (12228)	II	1.2×10^7	0/10
" " "	III	4.2×10^6	0/10
Streptococcus faecalis (19433)	II	9.9×10^6	0/10
" " "	III	2.3×10^7	0/10

Test Organism	Test Batch	Viable Count Per Carrier	Positive/Total Carriers
Streptococcus pyogenes (19615)	II	1.3×10^5	0/10
" " "	III	5.1×10^4	0/10
Staphylococcus aureus (MRSA-C.I.)	II	4.1×10^6	0/10
" " "	III	2.9×10^6	0/10
Candida albicans (C.I.)	II	2.4×10^5	0/10
" " "	III	1.5×10^5	0/10
Candida parapsilosis (C.I.)	II	3.0×10^5	0/10
" " "	III	1.6×10^5	0/10

9. Conclusions: Satisfactory performance vs. all tested organisms at a 1:256 dilution with 5% blood serum and 400 ppm $CaCO_3$ hard water at a contact time of 10 minutes.

   However, the test report did not specify any procedure used to insure neutralization of the germicide in subcultures.

b. Other AOAC Tests

1. AOAC Fungicidal Test

   i. Modifications: Organic soil load (5% blood serum added to fungal inoculum) and hard water (use-dilution made in 400 ppm $CaCO_3$ hard water.

   ii. Samples: LpH-SE/LpH-AG, Batch Nos. 673-16C2 and 673-16C3 (both received 03-22-85).

   iii. Dilution: 1:256.

   iv. Exposure: 5, 10, and 15 minutes (at 20C, per method).

   v. Test Organism: Trichophyton mentagrophytes ATCC 9533 (PR 1:60).

   vi. Subculture Medium/Neutralizer: Not reported.

   vii. Incubation: 25-30C for 10 days (per method).

   viii. Results:

Test Batch	Growth (+) or No Growth (-) at 5 min.	10 min.	15 min.
2-(60-Day)	-	-	-
"	-	-	-
3	-	-	-
"	-	-	-

ix. Conclusions: Satisfactory performance vs. T. mentagrophytes at a 1:256 dilution with 5% blood serum and 400 ppm $CaCO_3$ hard water at a contact time of at least 5 minutes.

However, the test report did not specify the subculture medium/neutralizer employed in the tests, or any procedure used to insure neutralization of the germicide in subcultures.

2. AOAC Tuberculocidal Activity Method (Confirmative In Vitro Test)

i. Modifications: Organic soil load (5% blood serum added to bacterial inoculum) and hard water (use-dilution made in 400 ppm $CaCO_3$ hard water.

ii. Samples: LpH-SE/LpH-AG, Batch Nos. 673-16C2, 673-16C3, and 673-16C4 (all received 03-22-85).

iii. Dilution: 1:256.

iv. Exposure: 10 minutes at 20C (per method).

v. Test Organism: Mycobacterium bovis (BCG) (per method).

vi. Subculture Medium/Neutralizer: Not reported.

vii. Incubation: 90 days at 37C (per method).

viii. Results:

Test Batch	Dilution	Positive/Total Carriers
673-16C2	1:256	0/10
673-16C3	"	0/10
673-16C4	"	0/10
Phenol	1:50	0/10
Phenol	1:75	5/10

ix. Conclusions: Satisfactory performance vs. M. bovis (BCG) at a 1:256 dilution with 5% blood serum and 400 ppm $CaCO_3$ hard water at a contact time of 10 minutes.

However, the test report did not show the results for each of the subculture media employed with the germicide or the phenol resistance controls.

3. AOAC Germicidal and Detergent Sanitizers Method

i. Modifications: Hard water (use-dilution made in 500 ppm $CaCO_3$ hard water).

ii. Samples: LpH-SE/LpH-AG, Batch Nos. 673-16C1, 673-16C2, and 673-16C4 (all received 08-14-85).

iii. Dilution: 1:256.

iv. Exposure: 30 and 60 seconds at 25C.

v. Test Organisms: Staphylococcus aureus ATCC 6538 (PR 1:65) and Escherichia coli ATCC 11229 (PR 1:95).

vi. Subculture Medium/Neutralizer: Letheen neutralizer blanks and tryptone glucose extract agar w/letheen (per method).

vii. Incubation: 48 hours at 37C.

viii. Results:

Test Organism	Numbers Control (cfu/ml)	Test Batch	Numbers Recovered 30 sec.	Numbers Recovered 60 sec.	Reduction (%) 30 sec.	Reduction (%) 60 sec.
S. aureus	75×10^6	673-16C1	0	0	100	100
" "	"	673-16C2	0	0	100	100
" "	"	673-16C4	0	0	100	100
E. coli	76×10^6	673-16C1	0	0	100	100
" "	"	673-16C2	0	0	100	100
" "	"	673-16C4	0	0	100	100

ix. Conclusions: Satisfactory performance vs. both test organisms at a 1:256 dilution in 400 ppm $CaCO_3$ hard water at a contact time of 30 seconds.

c. Virucidal Tests

1. Procedure: Two-tents ml of virus pool was spread over surface of glass petri dish and allowed to dry for 30-45 minutes at 35C. Then 2.0 ml of germicide at use-dilution was spread over the surface and allowed to remain for 10 minutes at 20-25C. After exposure, the virus-germicide mixture was removed by pipette and diluted in trypticase soy broth. Decimal dilutions were then inoculated into cell cultures or embroyanated eggs. After incubation at 35-37C for appropriate time, the presence or absence of virus was determined by cytopathogenic effect in cell cultures or by hemagglutination of chick or guinea pig erythrocytes from embroyanated egg cultures. The

virus control consisted of an inoculated surface exposed to 2.0 ml trypticase soy broth. The cytotoxicity control consisted of an uninoculated surface exposed to 2.0 ml germicide at use-dilution.

2. Modifications: Organic soil load (virus pool suspended in 100% chorioallantoic fluid or 5% calf serum) and hard water (use-dilution made in 400 ppm $CaCO_3$ hard water).

3. Samples: LpH-SE/LpH-AG, Batch Nos. 673-16C2 and 673-16C3 (both received 06-04-85).

4. Dilution: 1:256.

5. Exposure: 10 minutes at 20-25C.

6. Test Viruses: Influenza A2/Japan/305/57 (Host - 9-10 day chick embroyos), Herpes simplex Type 2 ATCC VR-734 (Host - Hep-2 cells), Vaccinia (Host - MRC-5 cells), and Adenovirus Type 2 ATCC VR-846 (Host - Hep-2 cells).

7. Incubation: 2 days (influenza), 12 days (herpes), 7 days(vaccinia), or 8 days (adenovirus) at 35C.

8. Results:

Test Virus	Test Batch	ID-50 or LD-50 (-Log 10)			
		Virus Control	Virus-Germicide	Toxicity	Reduction
Influenza A2/Japan	1	6.5	0.5	0.5	6.0
	2	6.5	0.5	0.5	6.0
Herpes simplex Type 2	1	5.5	1.5	1.5	4.0
	2	5.5	1.5	1.5	4.0
Vaccinia	1	5.5	1.5	1.5	4.0
"	2	5.5	1.5	1.5	4.0
Adenovirus Type 2	1	4.5	1.5	1.5	3.0
	2	4.5	1.5	1.5	3.0

9. Conclusions: Satisfactory performance vs. all test viruses at a 1:256 dilution with 5% blood serum and 400 ppm $CaCO_3$ hard water at a contact time of 10 minutes.

TECHNICAL SUPPORT SECTION EFFICACY REVIEW - II

Disinfectants Branch

EPA Reg. No. or File Symbol 1043-OR & 1043-OE

Date Division Received 09-12-85

Data Accession No(s). 259760 & 259758

Product Manager No. 32 (Castillo)

Product Name LpH-AG & LpH-SE

Company Name Vestal Laboratories

202.0 Recommendations

202.1 Efficacy Supported by the Data

a. The submitted data by the AOAC Use-Dilution Method appear acceptable to support effectiveness of the formulation for "LpH-AG" and "LpH-SE" as a disinfectant (hospital use) against Staphylococcus aureus, Salmonella choleraesuis, and Pseudomonas aeruginosa, and disinfectant against the additional pathogens Acinetobacter calcoaceticus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa (ATCC 27853), Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Shigella flexneri, Shigella sonnei, Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis, Streptococcus faecalis, Streptococcus pyogenes, Staphylococcus aureus (MRSA), Candida albicans, and Candida parapsilosis at a dilution of 1:256 in the presence of 5% blood serum and 400 ppm $CaCO_3$ hard water on moderately soiled hard, non-porous surfaces which are thoroughly wet for a contact time of 10 minutes.

However, the test reports entitled "Basic Bacteriological Data" and "Microbiological Data" did not specify the procedure used to insure that neutralization of the germicide in subcultures was achieved. In addition, these test reports did not include identification of the testing laboratory or the name(s) of the person(s) responsible for the tests. This information must be submitted to complete the above reports as indicated in 202.2 below.

b. The submitted data by the AOAC Fungicidal Test appear acceptable to support effectiveness of the formulation as a fungicide (pathogenic fungi) against Trichophyton mentagrophytes at a dilution of 1:256 in the presence of 5% blood serum and 400 ppm $CaCO_3$ hard water on moderately soiled hard, non-porous surfaces which are thoroughly wet for a contact time of 10 minutes.

However, the test report entitled "AOAC Methods of Analysis - Fungicidal Test" did not specify the subculture medium/neutralizer employed in the tests, or the procedure used to insure neutralization of the germicide in subcultures was achieved. This information must be submitted to complete the test report as indicated in 202.2 below.

c. The submitted data by the AOAC Tuberculocidal Activity Method appear acceptable to support effectiveness of the formulation as a tuberculocide against Mycobacterium bovis at a dilution of 1:256 in the presence of 5% blood serum and 400 ppm $CaCO_3$ hard water on moderately soiled hard, non-porous surfaces which are thoroughly wet for a contact time of 10 minutes.

However, the test report entitled "Tuberculocidal Test" did not specify, or show the results for, each of the subculture media employed with the germicide or phenol resistance controls. This information must be submitted to complete the test report as indicated in 202.2 below.

d. The submitted data by the Virucidal Test Methods are acceptable to support effectiveness of the formulation as a virucide against Influenza A2/Japan, Herpes simplex Type 2, Vaccinia, and Adenovirus Type 2 at a dilution of 1:256 in the presence of 5% blood serum and 400 ppm $CaCO_3$ hard water on moderately soiled hard, non-porous surfaces which are thoroughly wet for a contact time of 10 minutes.

e. The submitted data by the AOAC Germicidal and Detergent Sanitizers Method indicate effectiveness of the formulation as a sanitizing rinse against Staphylococcus aureus and Escherichia coli at a dilution of 1:256 in the presence of 500 ppm $CaCO_3$ hard water on pre-cleaned food-contact surfaces which are thoroughly wet for a contact time of at least 1 minute.

However, the establishment of a food additive regulation by the Food and Drug Administration is required for the acceptance of these data to support a sanitizing claim for this formulation on food-contact surfaces. The reccomendation for a potable water rinse after the sanitizing treatment is no longer an alternative for this requirement.

202.2 Additional Data/Information Required

a. For the test reports entitled "Basic Bacteriological Data" and "Microbiological Data" (AOAC Use-Dilution Method), indicate the procedure used and results obtained to insure that neutralization of the germicide in subcultures was achieved. Refer to the attached DIS/TSS-2 enclosure, item 7. In addition, identify the testing laboratory and name(s) of the person(s) responsible for the tests. Refer to the attached DIS/TSS-3 enclosure, 1st paragraph.

b. For the test reports entitled "AOAC Methods of Analysis - Fungicidal Test" (AOAC Fungicidal Test) by Northview Laboratories, indicate the subculture medium/neutralizer employed in the tests, and the procedure used and results obtained to insure that neutralization of the germicide in subcultures was achieved. Refer to the attached DIS/TSS-3 enclosure, item (1)(g), and DIS/TSS-2 enclosure, item 7.

c. For the test reports entitled "Tuberculocidal Test" (AOAC Tuberculocidal Activity Method - Confirmative InVitro Test) by Northview Laboratories, identify, and show the results for, each of the subculture media employed with the germicide or phenol resistance controls. Refer to the attached DIS/TSS-3 enclosure, items (1)(f) and (1)(g).

d. Establishment of a food additive regulation under 21 CFR 178.1010 by the Food and Drug Administration is required for acceptance of data to support a sanitizing claim for this formulation on food-contact surfaces

203.0 Labeling

a. In lieu of a food additive regulation by the Food and Drug Administration for the use of this formulation as a sanitizer for food-contact surfaces, this claim and pattern of use must be deleted. The recommendation of a potable water rinse after sanitizing is no longer an alternative for this requirement.

b. The pattern of use for this formulation as a disinfectant for food-contact surfaces, followed by a potable water rinse, is considered acceptable at this time. However, since this formulation and the pattern of use as a disinfectant are not cleared for food-contact surfaces, evidence to show the level of chemical residues on the treated surfaces may be required in the future.

c. On the right panel, "all" in the phrase "all washable hard non-porous surfaces" must be delted since it is too inclusive and exclusive of none.

TECHNICAL SUPPORT SECTION EFFICACY REVIEW - II

Disinfectants Branch

EPA Reg. No. or File Symbol 1043-OR & 1043-OE

Date Division Received 09-12-85

Data Accession No(s). 259760 & 259758

Product Manager No. 32 (Castillo)

Product Name LpH-AG & LpH-SE

Company Name Vestal Laboratories

EFFICACY DATA REQUIREMENTS

Supplemental Recommendations

When an antimicrobial Agent is intended for a use pattern that is not reflected by the test conditions specified in the Recommended Methods, one or more test conditions specified in the method must be modified and/or supplementary data developed in order to provide meaningful results relative to the conditions of use. The following basic information is critical to the development and submission of appropriate data.

1. EXPOSURE PERIOD

All products tested by the recommended methods may be tested at the exposure periods prescribed in those methods. However, if the product is intended for use at exposure periods shorter or longer than those specified in the method, the method must be modified, in a manner acceptable to the Agency, to reflect the deviation in exposure intended. A modification to provide a shorter exposure period is restricted by the manipulative limitations inherent in the method, while a modification to provide a longer exposure period is restricted by the conditions applicable to the use pattern. If a ten-minute exposure period is necessary for the antimicrobial agent to be effective against the test microorganism the product cannot be represented as an "instantly active" product, or cannot be represented as being "effective in 30 seconds,""one minute," or at any time period shorter than 10 minutes. Also, the product cannot be recommended for use in a manner which is inconsistent with the exposure period necessary for effectiveness (as, for example, "Spray on surface, and immediately wipe with clean cloth") unless the standard method has been modified and reflects efficacy under such conditions of use. In any case, the exposure period or manner of use necessary to provide efficacy must be featured prominently on the product label.

2. TYPE OF SURFACE

When an antimicrobial agent is intended to be effective in treating a hard porous surface, some of the Recommended Methods may be modified to simulate this more stringent condition by substitution of a porous surface carrier (such as a porcelain penicylinder or unglazed ceramic tile) for the non-porous surface carrier (stainless steel cylinder or glass slide) specified in the method. In addition, control data, described below in Supplemental Recommendation No. 6, must be developed to assure the validity of the test results when this modification of the method is employed. In no case may a surface carrier which represents a less stringent condition be substituted for a surface carrier which is specified in the Recommended Method.

3. HARD WATER

The Recommended Methods may be modified to demonstrate the effectiveness of an antimicrobial agent in hard water. The hard water tolerance level may differ with level of antimicrobial activity claimed. To establish disinfectant efficacy in hard water, all microorganisms (bacteria, fungi, viruses) claimed to be controlled must be tested by the appropriate Recommended Method at the same hard water tolerance level.

4. ORGANIC SOIL

An antimicrobial agent identified as a "one-step" cleaner-disinfectant, cleaner-sanitizer, or one intended to be effective in the presence of organic soil must be tested for efficacy by the appropriate method(s) which have been modified to include a representative organic soil such as 5% blood serum. A suggested procedure to simulate in-use conditions where the antimicrobial agent is intended to treat dry inanimate surfaces with an organic soil load involves contamination of the appropriate carrier surface with each test microorganism culture containing 5% v/v blood serum (e.g., 19 ml test microorganism culture + 1 ml blood serum) prior to the specified carrier-drying step in the method. Control data, described below in Supplemental Recommendation No. 6, must also be developed to assure the validity of the test results when this modification is incorporated into the method. The organic soil level suggested is considered appropriate for simulating lightly or moderately soiled surface conditions. When the surface to be treated has heavy soil deposits, a cleaning step must be recommended prior to application of the antimicrobial agent. The effectiveness of antimicrobial agents must be demonstrated in the presence of a specific organic soil at an appropriate concentration level when specifically claimed and/or indicated by the pattern of use. A suggested procedure for incorporating organic soil load where the antimicrobial agent is not tested against a dry inanimate surface, such as the AOAC Fungicidal Test, involves adding 5% v/v blood serum directly to the test solution (e.g., 4.75 ml test solution + 0.25 ml blood serum) before adding 0.5 ml of the required level (5×10^6 /ml) of conidia.

5. RE-USE

The Recommended Methods are designed to demonstrate efficacy of a freshly prepared antimicrobial solution intended for a single application. When the same use solution is intended for repeated applications, testing must be conducted in accordance with a test protocol specially designed to demonstrate retention of the claimed level(s) of antimicrobial activity in the use solution after repeated microbial and other appropriate challenges (such as supplemental recommendations indicated above) and stress conditions (such as an inadvertant or incidental dilution inherent in the use pattern) over the period of time or number of times specified in the directions for use.

6. MICROORGANISM SURVIVAL AFTER DRYING ON A HARD SURFACE

Quantitative determinations of the viable microbial concentration on the untreated control carrier after drying are required in order to determine the validity of the test results obtained with treated carriers when the Recommended Methods are modified to include such elements as (i) test microorganisms not specified in the method, (ii) substitution of a porous surface (e.g., porcelain penicylinder, unglazed ceramic tile) for the specified nonporous surface (stainless steel cylinder, glass slide), and/or (iii) an organic soil load. The detailed protocol for this testing must include: (i) preparation of inoculum, (ii) application of inoculum to the carrier, (iii) the time/temperature and relative humidity conditions for drying the microorganisms on the carrier, (iv) the technique for removal of the microorganisms from the carrier, and (v) the specific assay procedure indicating such details as replication, subculture media/diluents, and the incubation time/temperature conditions for the enumeration procedure employed. The test results must include the individual counts obtained by the method.

7. NEUTRALIZATION

For each antimicrobial product, procedures must be employed that will preclude residual effects of the active ingredient(s) in the subculture medium. A specific medium capable of neutralizing the antimicrobial effects of a product (whenever one is known) should be employed prior to the microbiological assay. Some of the Recommended Methods rely solely upon the selection of an appropriate subculture medium to neutralize the antimicrobial effects of certain general types of chemical compounds (active ingredients). However, to document absence of residual effects of the active ingredient(s) in the subculture medium, the following testing is necessary: (i) secondary subcultures must be performed to demonstrate that antimicrobial effects were overcome, or (ii) at the conclusion of the incubation period specified or employed in the method, the primary culture medium with test carrier must be inoculated with approximately 10 microorganisms/ml of the specific culture under test (documented by actual plate counts) and reincubated for the specified period to demonstrate that the subculture medium was capable of supporting bacterial growth.

8. BATCH REPLICATION FOR MODIFIED TESTS

Where the required batch replication has already been performed and accepted for a product registration with unmodified tests by the Recommended Methods, additional testing at the same use concentration under modified conditions (e.g., different exposure period, presence of organic soil or hard water, porous surface carrier, etc.) may be conducted with reduced batch replication, as follows: (i) for basic efficacy claims (e.g., sterilizers, disinfectants, or sanitizers), 2 samples, representing 2 different batches, instead of 3, and (ii) for supplemental efficacy claims (e.g., fungicides, virucides, or tuberculocides), one sample instead of 2.

202.0 Recommendations

202.1 Efficacy Supported by the Data

a. The submitted data by the AOAC Use-Dilution Method appear acceptable to support effectiveness of the formulation for "LpH-AG" and "LpH-SE" as a disinfectant (hospital use) against Staphylococcus aureus, Salmonella choleraesuis, and Pseudomonas aeruginosa, and disinfectant against the additional pathogens Acinetobacter calcoaceticus, Citrobacter freundii, Enterobacter aerogenes, Enterobacter cloacae, Escherichia coli, Klebsiella pneumoniae, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa (ATCC 27853), Salmonella typhi, Salmonella typhimurium, Serratia marcescens, Shigella flexneri, Shigella sonnei, Staphylococcus aureus (ATCC 25923), Staphylococcus epidermidis, Streptococcus faecalis, Streptococcus pyogenes, Staphylococcus aureus (MRSA), Candida albicans, and Candida parapsilosis at a dilution of 1:256 in the presence of 5% blood serum and 400 ppm $CaCO_3$ hard water on moderately soiled hard, non-porous surfaces which are thoroughly wet for a contact time of 10 minutes.

However, the test reports entitled "Basic Bacteriological Data" and "Microbiological Data" did not specify the procedure used to insure that neutralization of the germicide in subcultures was achieved. In addition, these test reports did not include identification of the testing laboratory or the name(s) of the person(s) responsible for the tests. This information must be submitted to complete the above reports as indicated in 202.2 below.

b. The submitted data by the AOAC Fungicidal Test appear acceptable to support effectiveness of the formulation as a fungicide (pathogenic fungi) against Trichophyton mentagrophytes at a dilution of 1:256 in the presence of 5% blood serum and 400 ppm $CaCO_3$ hard water on moderately soiled hard, non-porous surfaces which are thoroughly wet for a contact time of 10 minutes.

However, the test report entitled "AOAC Methods of Analysis - Fungicidal Test" did not specify the subculture medium/neutralizer employed in the tests, or the procedure used to insure neutralization of the germicide in subcultures was achieved. This information must be submitted to complete the test report as indicated in 202.2 below.

c. The submitted data by the AOAC Tuberculocidal Activity Method appear acceptable to support effectiveness of the formulation as a tuberculocide against Mycobacterium bovis at a dilution of 1:256 in the presence of 5% blood serum and 400 ppm $CaCO_3$ hard water on moderately soiled hard, non-porous surfaces which are thoroughly wet for a contact time of 10 minutes.

However, the test report entitled "Tuberculocidal Test" did not specify, or show the results for, each of the subculture media employed with the germicide or phenol resistance controls. This information must be submitted to complete the test report as indicated in 202.2 below.

d. The submitted data by the Virucidal Test Methods are acceptable to support effectiveness of the formulation as a virucide against Influenza A2/Japan, Herpes simplex Type 2, Vaccinia, and Adenovirus Type 2 at a dilution of 1:256 in the presence of 5% blood serum and 400 ppm $CaCO_3$ hard water on moderately soiled hard, non-porous surfaces which are thoroughly wet for a contact time of 10 minutes.

e. The submitted data by the AOAC Germicidal and Detergent Sanitizers Method indicate effectiveness of the formulation as a sanitizing rinse against Staphylococcus aureus and Escherichia coli at a dilution of 1:256 in the presence of 500 ppm $CaCO_3$ hard water on pre-cleaned food-contact surfaces which are thoroughly wet for a contact time of at least 1 minute.

However, the establishment of a food additive regulation by the Food and Drug Administration is required for the acceptance of these data to support a sanitizing claim for this formulation on food-contact surfaces. The reccomendation for a potable water rinse after the sanitizing treatment is no longer an alternative for this requirement.

202.2 Additional Data/Information Required

a. For the test reports entitled "Basic Bacteriological Data" and "Microbiological Data" (AOAC Use-Dilution Method), indicate the procedure used and results obtained to insure that neutralization of the germicide in subcultures was achieved. Refer to the attached DIS/TSS-2 enclosure, item 7. In addition, identify the testing laboratory and name(s) of the person(s) responsible for the tests. Refer to the attached DIS/TSS-3 enclosure, 1st paragraph.

b. For the test reports entitled "AOAC Methods of Analysis - Fungicidal Test" (AOAC Fungicidal Test) by Northview Laboratories, indicate the subculture medium/neutralizer employed in the tests, and the procedure used and results obtained to insure that neutralization of the germicide in subcultures was achieved. Refer to the attached DIS/TSS-3 enclosure, item (1)(g), and DIS/TSS-2 enclosure, item 7.

c. For the test reports entitled "Tuberculocidal Test" (AOAC Tuberculocidal Activity Method - Confirmative InVitro Test) by Northview Laboratories, identify, and show the results for, each of the subculture media employed with the germicide or phenol resistance controls. Refer to the attached DIS/TSS-3 enclosure, items (1)(f) and (1)(g).

d. Establishment of a food additive regulation under 21 CFR 178.1010 by the Food and Drug Administration is required for acceptance of data to support a sanitizing claim for this formulation on food-contact surfaces

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, D.C. 20460

EFFICACY DATA REQUIREMENTS

Reporting of Data

Systematic and complete descriptions of the tests employed and the results obtained are essential for proper review and evaluation of product performance by the Agency. All test reports must include identification of the testing laboratory or organization, when and where the tests were conducted, and the name of the person(s) responsible for the conduct of the tests.

(1) Recommended Methods. When the Recommended Methods (such as standard AOAC tests) are employed to develop efficacy data, certain minimal information must be provided in the test report. The report must include, but is not limited to, the following:

(a) Test employed, and any modifications thereto;
(b) Test microorganisms employed, including identification of the specific strain (ATCC or other);
(c) Concentration or dilution of product tested and how prepared;
(d) Number of samples, batches, and replicates tested;
(e) Preparation date of each product batch (individually formulated preparation of the product;
(f) Phenol resistance of test microorganisms (actual test results);
(g) Identification of all material or procedural options employed, where such choice is permitted or recommended in the test method selected (for example, growth media, drying time for inoculated carriers, neutralizer and/or subculture media, secondary subculturing);
(h) Complete report of results obtained for each individual replication;
(i) Any control data essential to establish the validity of the test.

(2) Modification of Recommended Methods. Where Recommended Methods are significantly modified to support specific claims and/or use patterns for a product, the protocol employed for modifying the test must be provided in specific detail with the test report. The applicant may submit the proposed modification for review and evaluation prior to initiation of the test.

(3) Other Methods. When Recommended Methods, or modification thereto, are not employed to develop efficacy data (such as actual in-use or many kinds of simulated-use testing), complete testing protocols must be submitted with the test reports. All materials and procedures employed in testing must be described in a manner consistent with original research reports published in technical or scientific journals. Where references to published reports or papers are made, copies or reprints of such references should be provided with the test reports. Proposed testing protocols for in-use or simulated-use studies of this kind may be submitted for review and evaluation by the Agency prior to initiation of the tests.

203.0 Labeling

a. In lieu of a food additive regulation by the Food and Drug Administration for the use of this formulation as a sanitizer for food-contact surfaces, this claim and pattern of use must be deleted. The recommendation of a potable water rinse after sanitizing is no longer an alternative for this requirement.

b. The pattern of use for this formulation as a disinfectant for food-contact surfaces, followed by a potable water rinse, is considered acceptable at this time. However, since this formulation and the pattern of use as a disinfectant are not cleared for food-contact surfaces, evidence to show the level of chemical residues on the treated surfaces may be required in the future.

c. On the right panel, "all" in the phrase "all washable hard non-porous surfaces" must be delted since it is too inclusive and exclusive of none.